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SEMIPERMEABILITY OF SEED COATS

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(WITH NINE FIGURES)

I. Introduction

As our knowledge of the physical and chemical characteristics of seed coats increases, the importance of the physiological rôle and the biological significance of these structures become more and more apparent. Much work has been done upon the germination of seeds without an adequate knowledge of the real conditions offered to the embryos for their development. Many chemical substances and various ethereal stimuli have been used to influence the germination of seeds, and as a rule the seeds have been used with coats intact. This has been true especially of the Germans who belong to the vitalistic school. The assumption has been that the coats of seeds, and cell walls generally, are permeable to practically all water-soluble substances, and that the dead membranes and cell walls do not modify ethereal and chemical stimuli which act through them, simply because they are dead.

Recently a number of papers have appeared which have a very important bearing upon these problems. In 1907 BROWN (10) reported the discovery of a semipermeable membrane forming the outer layer of the seeds of *Hordeum vulgare* var. *coerulescens*, and later (11) published an account of the selective permeability of this outer dead membrane of the seed. This work was followed by



SCHRÖDER (33), who found the same kind of selectively permeable coat in wheat. ATKINS (5) failed to find this character in the coats of beans. He found that the absorption of water from living and dead seeds was identical until germination commenced, at which time the osmotic phenomena of the living cells were manifested. The forces concerned in the initial stages of water intake are, according to ATKINS, those of capillarity and imbibition; but on germination, osmotic pressure begins to influence the amount of water taken up by the living seeds. As SCHRÖDER has pointed out (33, p. 188, footnote), ATKINS failed to take into account the open micropyle of the Leguminosae used; but the same seeds on moist sand, with the micropyle turned up, absorbed 90 per cent of their dry weight from 10 per cent NaCl in 6 days, according to SCHRÖDER. And since the present paper was written, TJEBBES (35) has found that the seeds of the sugar beet probably have a selectively semipermeable membrane as part of the inner seed coat.

Up to the present, therefore, semipermeable membranes have been reported only in the Gramineae and Chenopodiaceae. However, another interesting discovery was made in 1907 by BECQUEREL (7), who showed that the thoroughly dried seed coats of certain plants were impervious to various gases and to such penetrating substances as absolute alcohol, chloroform, and ether. He made no attempt to determine whether these coats were also semipermeable.

During the last two years I have been investigating the character of the seed coat of *Xanthium* with special reference to the work of BECQUEREL, BROWN, and SCHRÖDER, and present here the results of the work. I wish to acknowledge with thanks the encouragement and helpful advice of Dr. WILLIAM CROCKER, and to express my appreciation of the excellent facilities afforded me by the Hull Botanical Laboratory.

## II. Experimentation

### I. MOISTURE AND PERMEABILITY

The discovery by BECQUEREL that various gases, and alcohol, chloroform, and ether would not penetrate certain seed coats if completely dried, seemed so unusual that attempts were made to repeat his experiments, using the testa of *Xanthium glabratum*



cemented over the end of a glass tube fitted with a perforated rubber cork, the tube being then filled with mercury, and set up as a sort of barometer. Although the coats were supported by strong cloth, they were too delicate to withstand the strain, and would always burst on being inverted over the mercury cup. Shorter columns also were tried. In one case a column of mercury 20 cm. in height was sustained for four days without any diffusion of atmospheric gas taking place through the membrane.

Several attempts were made to test the diffusion of oxygen through the dry seed coat by means of an apparatus like that used by CROCKER (14) to test the diffusion through moist coats. These tests ran for only a few hours at a time and the results were negative. No measurable diffusion was noted, but the slightest injury to the coat was found to permit a rapid passage of oxygen.

Much better results were obtained with the experiments on the permeability of dry coats to ether, chloroform, acetone, and absolute alcohol. Seeds of *Xanthium glabratum* were dried for two weeks at 40° C., then put into a desiccator over phosphoric anhydride at 10 mm. atmospheric pressure for 18 days at 40° C., after which they were stored in a similar desiccator for use.

Water-free ether was prepared by treating the best ether obtainable with alcohol-washed KOH in sticks for 12 hours, and then distilling it with ribboned metallic sodium in both distilling and receiving flask. The sodium was allowed to act in the receiving flask until every trace of water had disappeared.

Five lots of dry seeds were then immersed in dry ether, and kept at a constant temperature in a Freas thermostat for various lengths of time from 2 to 36 days. On removing them from the ether the seeds were carefully dried, soaked in water, the testas removed, and the embryos placed in germinative conditions; 87 per cent of the seeds germinated, and the young plants were just as vigorous as the untreated controls. In those sets of seeds soaked for 16 and 36 days respectively, 100 per cent of both uppers and lowers germinated, and the average growth in length in 6 days was for the uppers 9.5 cm., for the lowers 11 cm.

Seeds carefully dried were also placed in chloroform, absolute alcohol, and acetone without the precaution of drying the fluids.



In all cases the dried seeds remained for many days (16-30) in the fluids without showing any injury, as shown by their normal germination afterward. That these fluids do not penetrate the testa can be demonstrated indirectly by using seeds with defective coats. In no instance were such seeds found to be viable after more than about 15 hours' immersion in absolute alcohol, ether, chloroform, or acetone. The length of time seeds with broken coats can remain alive in these substances depends upon the size and position of the defect. If the break occurs immediately over the hypocotyl, a few hours' immersion will kill the seeds; but if at the tips of the cotyledons, they will remain viable some hours longer.

The question naturally arises as to how much moisture can be put into these liquids, and still leave the seeds uninjured by long soaking. Commercial 95 per cent alcohol was found not to kill merely air-dried seeds in 4 days' time, but had killed them within 8 days. A series was then run in 90, 80, 70, 50, and 35 per cent alcohol. In a few hours the seeds in the lowest three grades had become excessively swollen and semitranslucent; they were found to be dead. Those in 80 per cent were by no means so swollen, but had been killed. Those in 90 per cent were apparently hard and sound, but at the end of 3 days all were dead. They may have been killed in considerably shorter time than this, as they were not tested for germination till the end of 3 days.

The results of these experiments confirm the findings of BECQUEREL, who kept various seeds in alcohol, ether, and chloroform for a full year without any serious loss of viability. While these facts seem remarkable, they merely confirm the results reported a good many years ago by GIGLIOLI (21), who obtained, for instance, the germination of 20 per cent of alfalfa seeds after more than 16 years in a saturated solution of  $\text{HgCl}_2$  in absolute alcohol. GIGLIOLI says that many of these seeds produced plants which flowered and fruited normally after this long exposure to destructive gases and liquids. The protective nature of the dry seed coats of certain seeds, therefore, is fully established, and is due to impermeability.

The results of these experiments offer no evidence against BECQUEREL's view that gases, especially oxygen, are not able to



diffuse through the thoroughly dried seed coats of certain seeds. CROCKER (14) was inclined to believe, from his study of the respiratory ratio in *Xanthium* seeds, that more oxygen diffused through the dry seed coats than through those which had not been thoroughly dried. This conclusion is probably shown now to be incorrect. The gas exchange noted by him in the case of dried coats may have occurred at least partially through defects in the coats; for, as I shall show later, there are a good many seeds which prove to have defects which are invisible, even on microscopic examination, and these defects may possibly be increased in number by the process of drying.

## 2. SELECTIVE SEMIPERMEABILITY OF XANTHIUM SEED COATS

A few preliminary tests to determine the amount and rate of imbibition of *Xanthium* seeds in solutions of sodium chloride of various strengths indicated the existence of a very efficient semipermeable membrane in the seed coat. Water enters the seed very rapidly under the powerful forces of capillarity and imbibition until more than 50 per cent of the air-dry weight of the seeds has been taken up. Nearly all of the water is taken up in 12–15 hours, as shown by the curves in figs. 1 and 2.

This rapid intake of water is in sharp contrast with that which occurs in the Gramineae. In the seeds of the latter the rate of water intake is considerably slower, and extends over 7 or 8 days or even longer, before saturation is reached. Evidently the protective structures influence the rate at which water passes into the seeds.

When strong salt solutions instead of distilled water are used with *Xanthium* seeds, the powerful internal forces, capillarity, imbibition, and perhaps the chemical

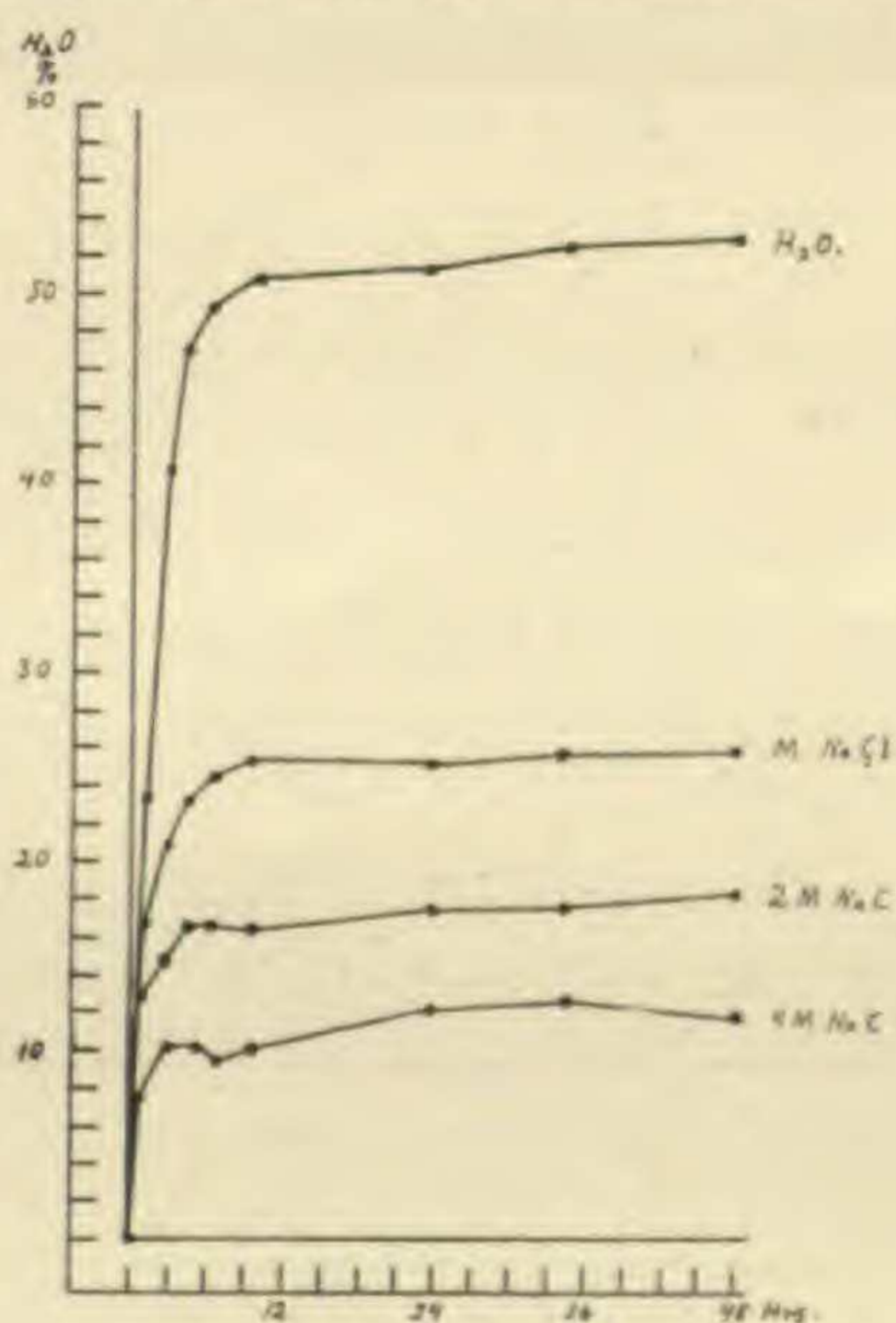


FIG. 1.—Curves of imbibition for seeds of *Xanthium* in salt solutions of various strengths, and in water; time element plotted on the axis of abscissae, percentage increase over air-dry weight on the axis of ordinates.



attraction of colloids, cause the water to enter with almost as great initial rapidity as when pure water is used, until the osmotic pressure of the salt on the outside balances the internal forces mentioned; then the entrance of water quickly ceases. After equilibrium is established in any given salt solution, increasing or decreasing the density of the solution extracts water from the seed or permits water to enter it in accordance with the direction of the disturbance. The adjustment continues rapidly after the disturbance until the equilibrium is reestablished, when the amount again remains constant.

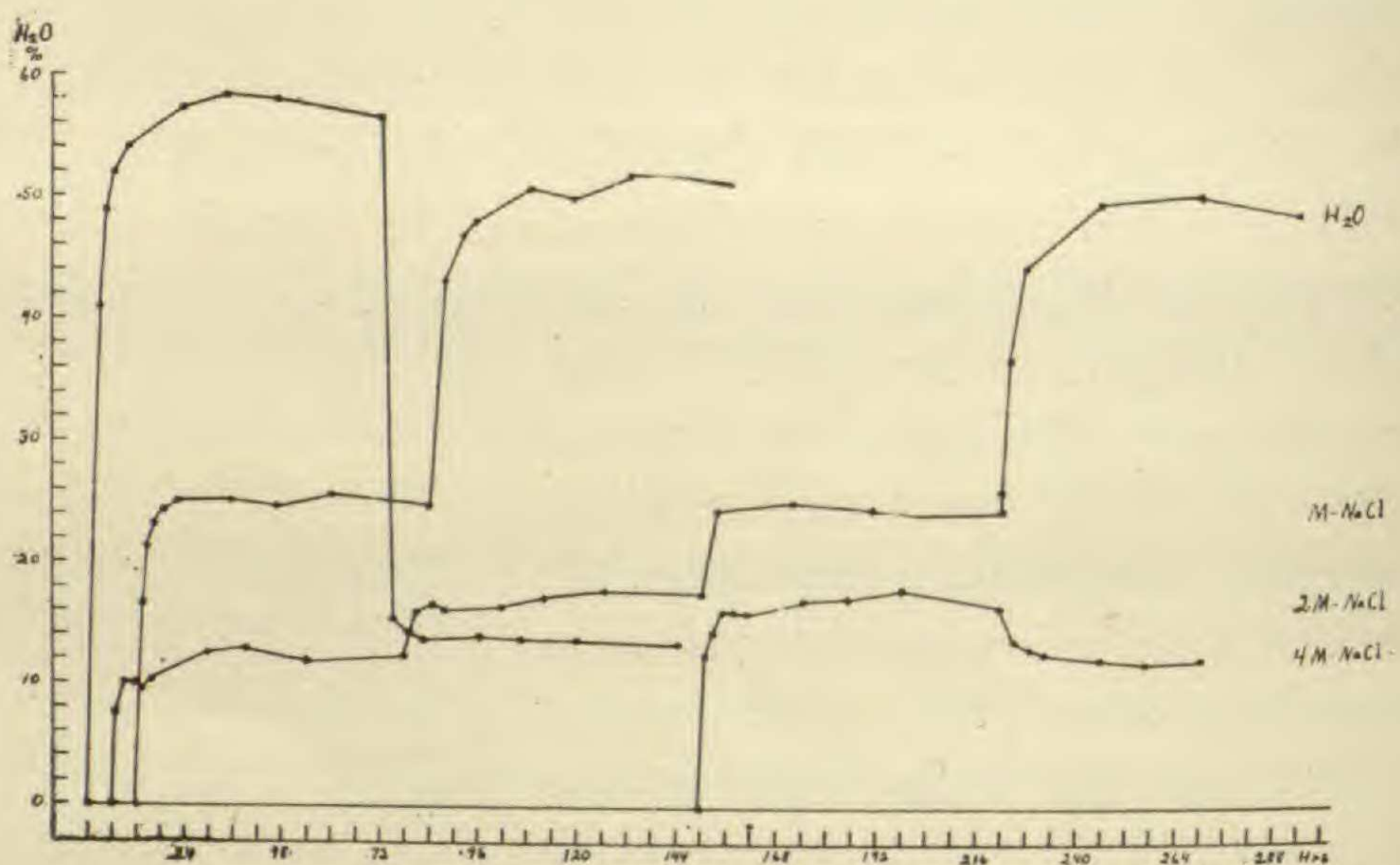


FIG. 2.—Curves showing entrance and withdrawal of water in *Xanthium* seeds on shifting from water to salt solutions, or vice versa, at three-day intervals; the semi-permeability of the membrane is well illustrated by the behavior recorded in these curves; curves 2, 3, and 4 are displaced to the right to avoid confusion.

The speed with which this adjustment of internal and external forces takes place in *Xanthium* is again in contrast with the rate of adjustment in grasses, such as wheat and barley, in which the rate of adjustment is slow, requiring days rather than hours to establish equilibrium. It appears that the coat in *Xanthium* offers very slight resistance to the passage of water, while in the Gramineae a comparatively much greater resistance is offered by the coat structures.

This relation of osmotic pressure to capillary and imbibition forces, chemical and physical, through the semipermeable coat of the *Xanthium* seed, is splendidly illustrated by figs. 1 and 2, which



show the imbibition curves for distilled water, and for molecular, two-molecular, and four-molecular solutions of NaCl as the seeds were shifted from one strength to another at three-day intervals. The close agreement of the constant portion of the curves in each strength of solution should be noted. The character of the curve during the last two days of each three-day interval indicates that the salt does not enter in appreciable amounts. Comparison with the type of curve given by SCHRÖDER (33, p. 189) for wheat shows that this membrane is at least as efficient in excluding NaCl as the coats of the Gramineae.

By appropriate chemical methods it has been shown that no passage of the salt through the membrane occurs. It was necessary, however, to test seeds singly, since one finds rather frequently seeds which have invisible defects in the coat which allow the passage of salts.

Many other chemical substances, usually in molecular solutions, were used to determine the range of selective semipermeability. The permeability or non-permeability to each substance was judged by the amount of imbibition water taken up from such solutions of acids, bases, and salts. Anyone who investigates the subject will be convinced that this means of determining semipermeability is sufficiently accurate for all ordinary purposes. The results, in percentage of increase over air-dry weight by imbibition, are given in table I.

From this table it is seen that the nitrates as a class, and especially silver nitrate, penetrate the coats. Iron sulphate slowly enters, while copper sulphate does not. The penetration of copper sulphate can be detected, if it occurs, by removal of the testa and examination of the embryo. Occasionally as high as 20-40 per cent of the seeds show slight localized penetration of  $\text{CuSO}_4$  through defects in the coat too slight to be seen on microscopic examination of the dry seeds. Because of these defects the figures given for any salt are approximations only; but in case there is general permeability it is easily recognized by the amount of imbibition. Since 60-80 per cent of the seeds show no penetration even after prolonged soaking in  $\text{CuSO}_4$ , and since they retain their vitality perfectly, notwithstanding the very highly poisonous character of



the solution, it seems reasonably certain that copper sulphate does not penetrate a sound testa.

TABLE I  
PERCENTAGE OF IMBIBITION  
(BASED ON AIR-DRY WEIGHT)

OSMOTIC PRESSURE (CALCULATED FROM THE LANDOLT- BÖRNSTEIN TABLES)* IN ATMOSPHERES	SUBSTANCE	END OF THREE DAYS		TEN DAYS	REMARKS	
		Exp. I	Exp. II	Exp. II		
	Water	54.94	53.95	.....	End of two days	
38.02.....	M-NaCl	26.	24.46	.....		
72.01.....	2M-NaCl	21.58	18.47	.....		
130.62.....	4M-NaCl	12.16	13.44	.....		
	Sat. NaCl	7.12	6.2	.....		
35.53.....	M-AgNO <sub>3</sub>	49.83	46.57	70.89		
37.66.....	M-NH <sub>4</sub> NO <sub>3</sub>	32.47	33.	40.6		
37.67.....	M-NaNO <sub>3</sub>	34.18	28.88	32.09		
36.53.....	M-KNO <sub>3</sub>	37.89	38.87	41.29		
28.25.....	M-CuSO <sub>4</sub>	34.48	35.4	35.59		
	M-FeSO <sub>4</sub>	34.39	37.48	44.73		
39.6.....	M-KCl	35.15	37.62	38.3		
	M-K <sub>2</sub> CrO <sub>4</sub>	24.55	32.04	36.91		
	M-Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	28.37	.....	.....		
	5% HgCl <sub>2</sub>	70.2	.....	.....		
	CH <sub>3</sub> OH	66.6	.....	.....		10 volume per cent
	C <sub>2</sub> H <sub>5</sub> OH	57.14	.....	.....		" " " "
	C <sub>3</sub> H <sub>7</sub> OH	69.25	.....	.....	" " " "	
	Glycerol	29.32	.....	.....	" " " "	
	Ether sat.	53.95	.....	.....	End of 5 hrs.	
	Iodine, 5 per cent in KI..	.....	.....	.....	Penetrates	
	M-Sucrose	34.41	.....	.....	In 23 hrs.	
	M-Fructose	35.54	.....	.....		
	M-Glucose	36.51	.....	.....		
	M-Lactose	35.47	.....	.....		
	M-KOH	79.	.....	.....		
	M/10 KOH	72.2	.....	.....		
	M-NaOH	102.06	.....	.....		
	M/10 NaOH	50.2	.....	.....		
	M-HCl	32.87	.....	.....		
	M-H <sub>2</sub> SO <sub>4</sub>	42.06	.....	.....		
	M-HNO <sub>3</sub>	73.33	.....	.....	In 48 hrs.	
	M-Tartaric	36.87	.....	.....	In 11 hrs.	
	M-Acetic	57.3	.....	.....		
	M-Lactic	55.84	.....	.....		
	M-Citric	37.06	.....	.....		

\* In these calculations from the Landolt-Börnstein tables correction is made only for dissociation. The osmotic pressure of 2M and 4M-NaCl is probably somewhat higher than here indicated, due to the formation of hydration compounds. Saturated NaCl is believed to exert 375 atmospheres of osmotic pressure.

Mercury bichloride enters the seed rapidly, as do also aqueous solutions of iodine in KI, the monatomic alcohols, ether, the alkalies,



and most of the acids, except perhaps hydrochloric and tartaric. The entrance of sulphuric is very slow. The triatomic alcohol, glycerol, does not enter, nor do the sugars.

The semipermeability is not dependent on any living cells in the membrane, but is purely a physical phenomenon. Boiling the seeds does not destroy this character of the coat, nor do such poisons as  $\text{HgCl}_2$ ,  $\text{AgNO}_3$ , iodine, the alcohols, ether, etc. Solutions of sodium chloride extract water rapidly from seeds which have imbibed almost 70 per cent of their weight in 5 per cent  $\text{HgCl}_2$ . The peculiarity of the testa must therefore depend upon the physical structure and chemical composition of the dead cell walls, and upon the relations it may assume toward the various solutes and solvents, not upon any vital conditions.

The seed coat of *Xanthium* possesses a great advantage over the membranes discovered by BROWN and SCHRÖDER in barley and wheat as an object of research; for in *Xanthium* the entire coat is easily removed after soaking for a few hours in water, and can then be used readily as an osmotic membrane.

For this purpose I have used the apparatus illustrated in fig. 3. A glass tube about 6 cm. in length and 1.5 cm. in diameter is closed at both ends with short rubber corks, each perforated by a hole 3 mm. in diameter. Since the corks must fit tightly into the glass tube, the perforation of one of the corks is prevented from collapsing by inserting a short piece of glass tubing 3 mm. in diameter. The testa is prepared for use by cutting it longitudinally along one side, and cutting off the ends. The testa can then be opened out and dried under a weight, thus producing a rectangular membrane about  $6 \times 8$  mm.

This membrane is cemented carefully to the rubber cork which has been prevented from collapsing by the narrow glass tubing. It is best to place a thin layer of wax over the cork first, then press the membrane firmly into the wax, after which the edges of the dry



FIG. 3.—Osmotic apparatus used in testing directly the semipermeability of *Xanthium* seed coats; description in text.



membrane are carefully sealed with more of the wax, leaving only that portion of the membrane immediately over the hole in the cork entirely free from wax. In my experiments the area of membrane left uncovered has been approximately equal to the area of the perforation through the cork.

The 6 cm. glass tube is now placed in vertical position, with the membrane at the lower end. A 4 M solution of NaCl is introduced through the upper cork until the chamber is quite filled. Care must be taken that small bubbles of air which tend to remain in the perforation of the lower cork while the chamber is being filled do not prevent the solution from filling this perforation. Otherwise the membrane will not be in contact with both water and solution.

Finally a long glass tube 3 mm. in diameter is carefully inserted through the upper cork. The salt solution will rise a short distance in the narrow tube, due to displacement. After making sure that the apparatus is entirely free from salt solution externally, the chamber is suspended in a vessel of distilled water with the liquids at the same level, just as if it were a thistle tube. This apparatus and the experiment itself are so simple that they should be of considerable pedagogical interest, as demonstrating directly the independence of semipermeability of this kind from any vital matter.

In one such experiment a rise of 175 mm. occurred in the narrow tube in 7 days. This is a fairly rapid rise, inasmuch as the tube in which the rise was measured had practically the same cross-section area as the diffusion membrane. It means that on the average a column of water 25 mm. long passed through the membrane in a day's time. The rate of passage is so rapid as to indicate a very slight resistance of the membrane to the passage of water, a condition quite different from that found in the Gramineae, as already suggested.

Here again the impermeability of the membrane was demonstrated by dropping some of the liquid taken from immediately below the diffusion membrane into  $\text{AgNO}_3$ . No visible precipitate was obtained, even at the end of 7 days.

### 3. STRUCTURE AND COMPOSITION OF THE TESTA

The morphological development of the seed coat of *Xanthium* has not been fully traced, but it is probable that the seed coat is



formed in the same way as in another of the Compositae, *Helianthus*, the structure of which has been reported by BRANDZA (9).

In *Helianthus* the inner layer of the 3-layered seed coat is formed from the nucellar epidermis, and is composed of just one layer of cutinized cells. *Xanthium* likewise has a 3-layered testa, the inner layer of which is a single layer of cells excepting over the hypocotyl, where, as CROCKER has shown, it is several cells thick. It is probable that this inner layer, with the exception of its chalazal portion, which lies immediately over the hypocotyl, is derived from the epidermis of the nucellus.

At first it was suspected that the inner stratum might be the semipermeable layer, although CROCKER had suggested in 1906 that the thicker middle one probably excluded oxygen. While attempt was being made to locate the seat of the semipermeability it was discovered that certain substances, the higher alcohols, acetic acid, and the alkalies, occasionally cause a bursting of the middle layer without injuring the single-celled inner lamina. The outer layer, of course, is too loose and chaffy to function osmotically.

In the case of M and M/10 solutions of KOH and NaOH, the bursting of the middle layer is followed by a periclinal separation of the inner layer from the middle one, which is probably the ovular integument, so that the embryo, surrounded by the intact, thin, delicate, one-celled inner layer can be removed from the external coats. This discovery was a very important aid in studying the semipermeability of the different layers of the testa, and illustrates the advantage which removable coats have over such as have been found in the grains. In the barley and wheat all such investigations had to be indirect, because the membrane could not be removed or separated into distinct layers.

The thin inner coat was shown to be a very efficient semipermeable membrane by treating the much swollen seeds surrounded by this membrane with 4M-NaCl. At the time one such seed was introduced into the salt solution it weighed 110 mg. After one hour the membrane was completely collapsed and pressed very tightly against the embryo. It weighed 58 mg. In 4 hours it weighed 52 mg., in 10 hours 51 mg., after which a rise in weight gradually occurred, probably due to a very slow entrance of NaCl.



In 2 days its weight was 59 mg. Fig. 4 shows the curve for loss of water from such a seed which weighed 198.5 mg. at the time it was put into NaCl.

The middle layer, which is several cells thick, was subjected to a direct test by using it as an osmotic membrane cemented over a rubber cork. Its osmotic character is demonstrated by the following figures:

Rise	Time	Rise	Time
45 mm.....	5 hrs.	159 mm.....	32 hrs.
72 " .....	9 "	169 " .....	48 "
125 " .....	20 "	171 " .....	72 "

However, this layer is by no means as efficient as the inner layer. The NaCl could be seen streaming through the membrane into the distilled water, causing the same appearance as the rapid solution of a salt in water.

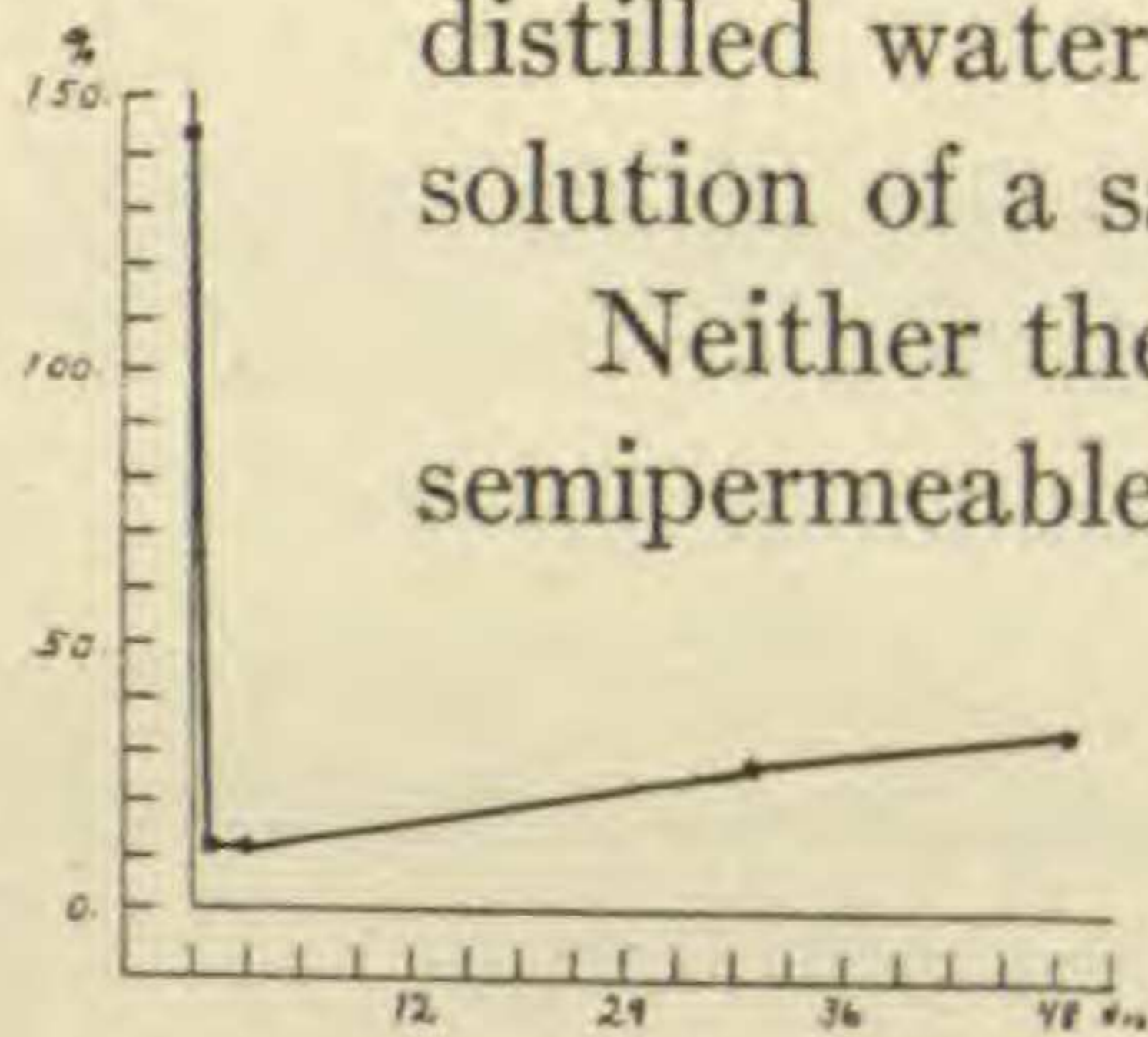


FIG. 4.—Curve showing loss of water by a seed which had become excessively swollen in M-NaOH when later put into 4M-NaCl; the outer coats of the testa had burst, leaving the inner coat, one cell thick, intact; the curve demonstrates the semipermeable character of the inner layer.

Neither the middle nor inner membrane is as perfectly semipermeable alone as are both together in the natural condition. Microchemical study of the coat, using NaCl and AgNO<sub>3</sub>, had given evidence in the same direction, that the middle layer allowed salts to reach the inner layer, but that the precipitate of AgCl was not certainly present within the inner layer. The extreme stretching of the coats preceding the bursting, or a physical or chemical modification due to the macerating agent, may be responsible for the impaired efficiency of the separated membranes.

The cellular arrangement in the inner membrane is shown in fig. 5. Microchemical tests showed the walls of this layer to be largely cellulose, with little or no suberization. A dark blue color was obtained on treatment with sulphuric acid and iodine, and the materials of which it is composed are almost completely dissolved in Schweizer's reagent. Practically no coloration was obtained with alkanin and other suberin reagents, except in the cell contents, and these are known not to be respon-



sible for this physical character of the membrane, which remains unchanged when the cell contents have been removed by dissolution.

REICHARD (29) some years ago showed that the coats of *Hordeum* are full of tannin, and has now (30) raised the question whether the tannin present there has any effect on the semipermeability of its membranes. The colloidal condition of the tannin, and its known peculiarities chemically, suggested that it might be in a large measure responsible for the physical properties of the coat. He investigated the position of the tannin by micro-chemical methods, and found that in sections the barley grains showed a sharply defined layer of tannin which could easily form a continuous coat. Treatment of seeds with tannin solvents showed that with such treatment the layer of tannin becomes broader and more diffused. Alcohol (96 per cent) used alone does not dissolve tannin. Following the alcohol treatment with iron sulphate solution as a tannin stain shows that the tannin layer becomes if anything more sharply defined in alcohol of high grade. But if the seeds are soaked first in water, then the tannin shows marked solution effects when subsequently treated with alcohol. Alcohol seems to dissolve tannin only when it has been previously wet.

According to REICHARD'S view, the entrance of substances into the seed would depend upon their ability to dissolve the tannin layer, or to dissolve in it. Since strong alcohol does not dissolve tannin, seeds with such a tannin layer might lie in absolute alcohol indefinitely without loss of vitality. But the presence of sufficient moisture to dissolve or partially dissolve the tannin would allow rapid entrance of alcohol, and a consequent killing of the embryo. The bearing of these facts upon the resistance of dry seeds to organic solvents discussed in an earlier section of this paper is obvious.

Hydrates, especially NaOH, have a powerful solvent effect upon tannin; even in M/10 solutions the solvent action is apparent, and

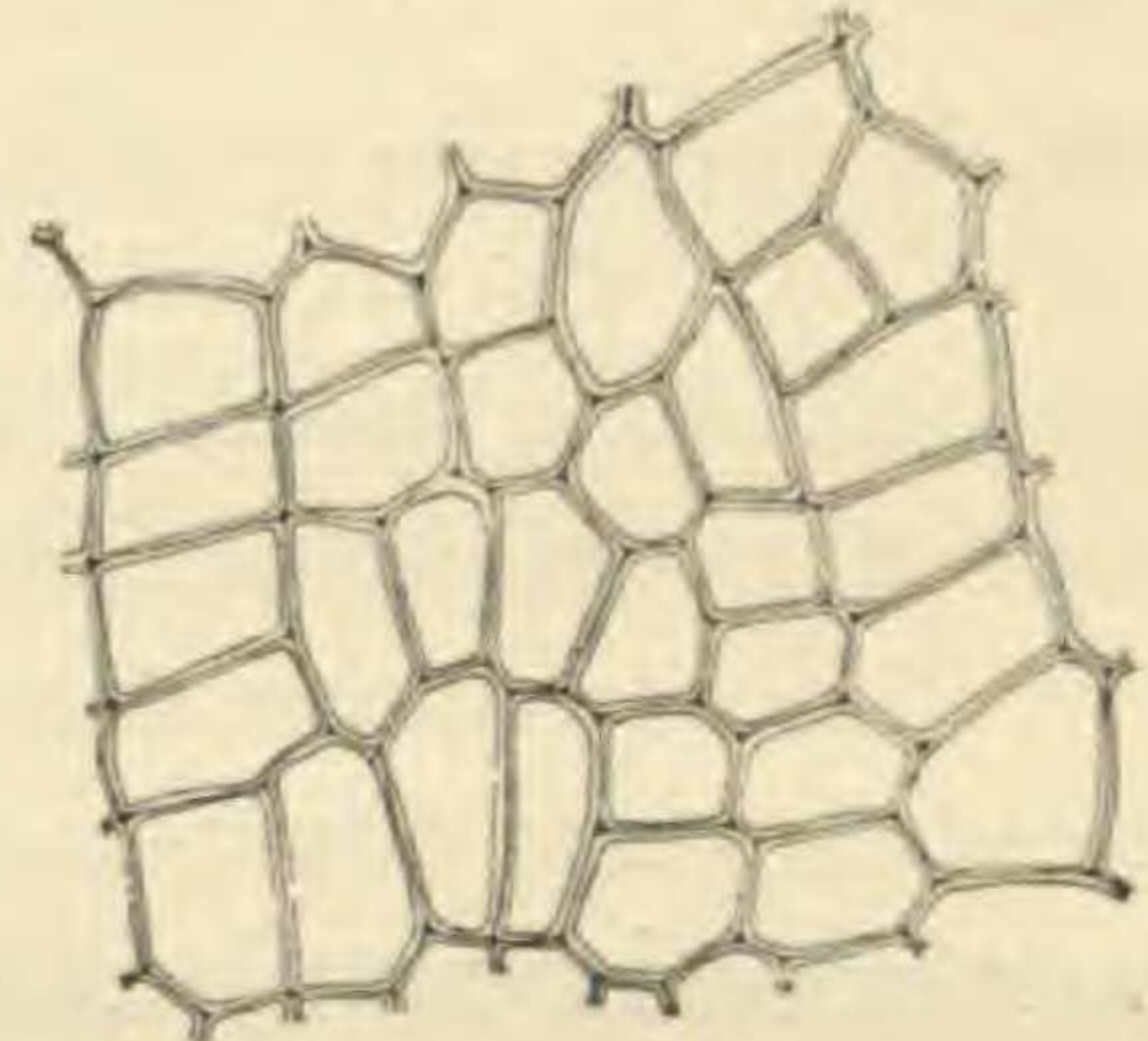


FIG. 5.—Cellular arrangement of a portion of the inner layer of the testa.—Camera lucida, 4 mm. obj., 4 ocular, 160 mm. tube.



as the concentration increases, the action is very rapid. On the other hand, acids do not dissolve tannins, but rather precipitate them from solution, according to REICHARD.

The barley grains used by REICHARD did not seem to be as perfectly semipermeable as those used by BROWN. This variation in semipermeability is attributed to differences in the tannin content from differences in the ripening process. He believes that the tannin is an "assimilation" product of chlorophyll, and that in unripe seeds the tannin has not been fully deposited in the seed coats. Or one can assume that the tannin for some unknown reason has failed to reach the proper chemico-physical condition for complete semipermeability.

While it seems entirely possible that the resistance of dry seeds to alcohol and other reagents such as ether, chloroform, etc., might be related in certain cases to tannin in the seed coats, it does not seem probable to me that semipermeability of these membranes can be explained on these grounds.

There is a considerable amount of tannin in the testa of *Xanthium*, as shown by treatment with ferric chloride, ferric sulphate, potassium dichromate, etc. But the tannin in this case does not seem to form so definite a layer as REICHARD reports for *Hordeum*. It seems to be scattered through the tissue in minute granules, occurring especially in the outer and middle layers of the coat with only very minute quantities in the inner layer. As has been shown, treatment of the coats with molecular NaOH, which is a strong tannin solvent, according to REICHARD, does not destroy its semipermeable character. For these reasons it is the writer's opinion that the property belongs to the cellulose, rather than to substances associated with it in the coat. Further microchemical studies upon the seed coat of *Xanthium* are in progress, and in time it may be possible to analyze the conditions which cause the exclusion of certain salts and the passage of others through the semipermeable coat of seeds.

#### 4. SEMIPERMEABILITY A WIDESPREAD PHENOMENON

In addition to the work on *Xanthium*, a number of seeds in various families have been tested. The results show that the



lifeless membranes forming the coats of seeds frequently exhibit osmotic effects. A number of widely separated families are represented in the list now known to possess such properties: Alismaceae (*Alisma Plantago-aquatica*), Gramineae (barley, wheat, oats, etc.; probably most grasses), Chenopodiaceae (sugar beet), Rosaceae (peach, apple), Leguminosae (*Vicia Faba*, scarlet runner, lima bean), Compositae (*Xanthium*, *Helianthus annuus*). It should be said at once that these seeds do not all exhibit the same degree of semipermeability. The coat of *Xanthium* apparently permits no passage or only very slow passage of certain solutes, comparing favorably with the coats of *Hordeum* and *Triticum* in this respect. Coats of the sunflower and peach are only less efficient, while the bean coats usually allow a noticeable passage of salts. The Leguminosae seem to be less uniform in their behavior than any seeds tested. Certain specimens have very good semipermeable membranes, others have rather poor ones.

As pointed out in the introduction, ATKINS found Leguminosae not to possess semipermeable testas, but had overlooked the open micropyles. Direct methods of testing proved that these membranes do act osmotically. It was not found practicable to wax these membranes onto rubber corks, because the great amount of expansion of the membrane on soaking, after the apparatus was arranged, always resulted in breaking the coat loose from the wax. To overcome this difficulty, the wet bean coats were placed tightly between two perforated rubber corks which were smeared slightly with vaseline, and used just as in the case of *Xanthium* already described. In this case the hole in the cork below the membrane must also be carefully filled with water to insure contact of both fluids with the membrane.

Coats of *Vicia Faba* used with saturated NaCl solution gave a rise of 72 mm. in 36 hours; and of scarlet runner a rise of 135 mm. in 10 days, the rise continuing throughout this time, but amounting to only 17 mm. during the last 4 days. The escape of the salt through the coats of both these legumes was readily demonstrated by use of  $\text{AgNO}_3$ .

It is a pleasure to record the fact that Professor STEVENS of the University of Kansas has for more than 8 years been using the coats



of the lima bean to demonstrate osmosis in his elementary classes in plant physiology. The coats are merely tied over the ends of glass tubes. His experience has been that the coats are irregular in their behavior, and that successful use demands the testing of the membranes, and the choice of those whose behavior with a definite strength of solution is uniform. Thus chosen, the membranes may be used to demonstrate admirably PFEFFER'S discovery that osmotic pressure varies directly with the concentration of the solution.

Recently LAVISON (24) studied the entrance of salts into the roots of plants and showed that the cellulose "frames" (*cadres*) around the cells of the endodermis are impermeable to certain salts, and that entering salts must therefore pass through protoplasm in penetrating the root beyond the endodermis. Later (25) he made the further observation that the cellulose cell walls forming the periclinal walls of the endodermis behave toward entering salts like the protoplasm itself. That is, those salts which are excluded by the protoplasm are excluded by the walls also. LAVISON does not say that the walls are selectively semipermeable, but that is seemingly the only possible interpretation of his observation. It should be noted that this applies only to the *young* cellulose walls.

All of this evidence points to semipermeability as a widespread phenomenon among lifeless plant membranes. Of course, the membrane must be permeable to water if it is to be osmotically active. The thin skin of potatoes is impermeable to water solutions, allowing neither salt nor water to pass through. The possibly semipermeable character of cellulose membranes cannot be overlooked in future investigations dealing with the entrance of salts into plant tissues.

##### 5. OSMOTIC PRESSURE AND IMBIBITION FORCES

The experiments upon *Xanthium* seeds with concentrated salt solutions suggested the possibility of measuring the capillary and imbibition forces of seeds by use of solutions of very high known osmotic pressure, since the balancing of osmotic pressure on the one hand against capillarity and imbibition on the other through a perfect semipermeable membrane is a very simple matter.

For this purpose saturated solutions of lithium chloride, which



gives the highest osmotic pressure of any known neutral salt, was employed. The imbibition curve for air-dry seeds known to contain 8-9 per cent of moisture is shown in fig. 6. In the case of both lower and upper seeds there was found a slight loss in weight during the first few hours of soaking in this concentrated LiCl solution. The loss is very small in amount, averaging 0.5 mg. per seed in all the tests made. But after the seeds have been in the solution for 6 or 7 hours, there is a very slow increase in weight, so that at the end of 46 hours the weight of the soaked seeds was the same as when first put into the fluid. The exact significance of this behavior is not very clear, but it is obvious that these seeds do not possess internal forces of sufficient magnitude to withdraw any water from the LiCl solution.



FIG. 6.—Imbibition curve for air-dry seeds in saturated LiCl solution.

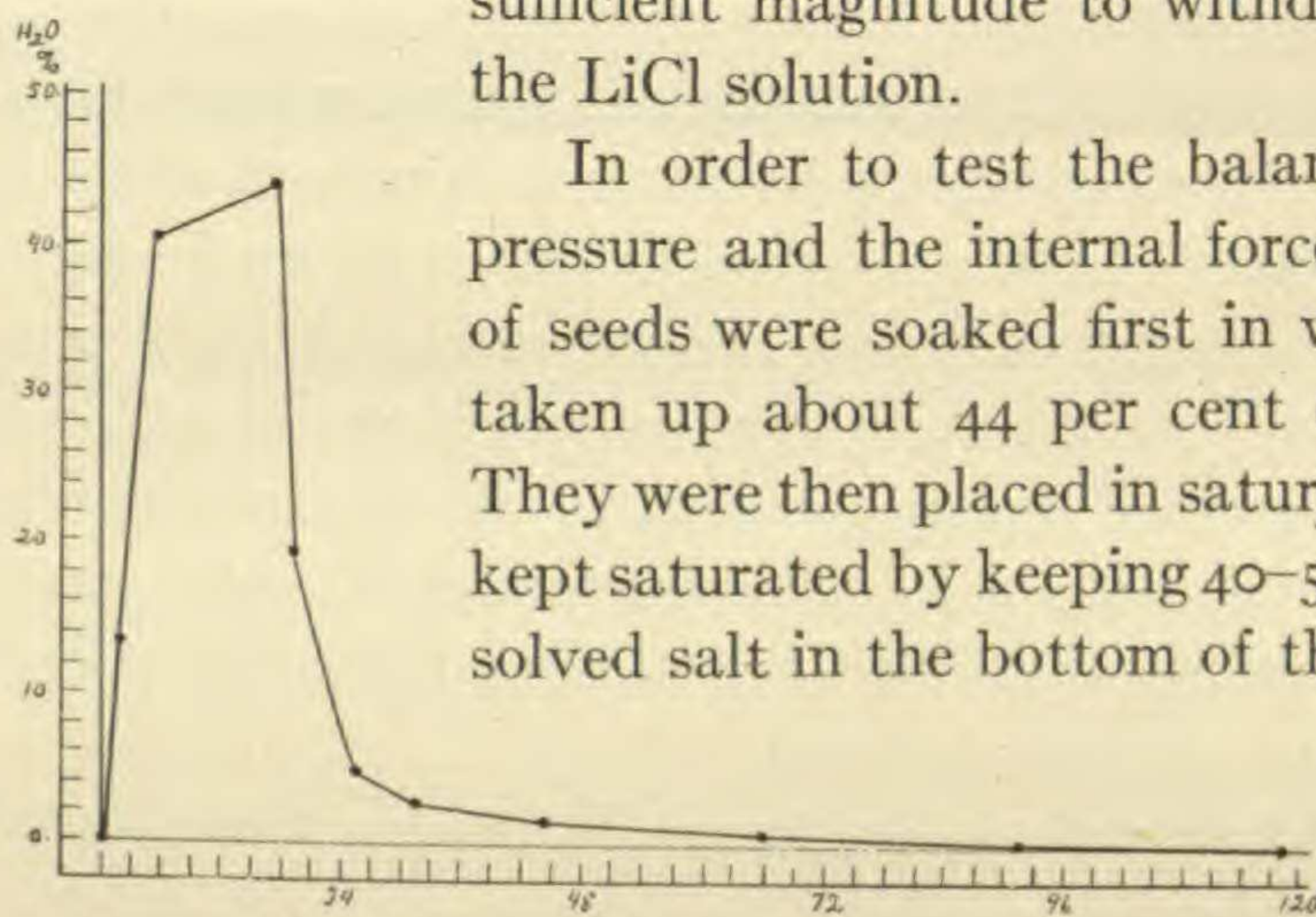


FIG. 7.—Curve showing loss of water from seeds soaked in water before transferring to saturated LiCl solution; imbibition force of air-dry seeds and osmotic pressure of saturated LiCl solution approximately equal.

In order to test the balance between osmotic pressure and the internal forces further, a number of seeds were soaked first in water until they had taken up about 44 per cent of their dry weight. They were then placed in saturated LiCl, which was kept saturated by keeping 40-50 grams of the undissolved salt in the bottom of the vessel and stirring

it up frequently. The imbibition curve is shown in fig. 7. At the end of one hour, more than three-fourths of the

water had been withdrawn, and in 7 hours the seeds were only 2.5 per cent above their air-dry weight. The water was withdrawn more and more slowly until at the end of 100 hours they reached their original dry weight. The results shown in figs. 6 and 7 indicate that capillarity and imbibition force in an air-dry seed of *Xanthium*, that is, one with 8-9 per cent of moisture, is approximately equal to the osmotic pressure of a saturated solution of LiCl.



One of the most difficult problems was to secure adequate data as to the size of the pressures and forces indicated by the results. The literature aside from the work of the physical chemists contains serious disagreements, and the physical chemists have done very little on the osmotic pressure of concentrated solutions. Moreover, there is wide divergence in the interpretation of the little they have done. As the writer is not a physical chemist he has been at a distinct disadvantage in the attempt to clarify a very confusing situation.

In his work on *Hordeum*, BROWN ascribes to a saturated solution of NaCl an osmotic pressure of 125 atmospheres. A few years earlier, RACIBORSKI (28), who investigated the upper limits of osmotic pressure in living plant cells, cites DIETERICI as authority for the statement that saturated NaCl has at 20° C. an osmotic pressure of 375 atmospheres. RACIBORSKI gives the pressure as 349.11 atmospheres at 0° C., the saturation concentration being 35.51 per cent. The figures given by BROWN are apparently based on the assumption that the law announced by VAN'T HOFF in 1887, that osmotic pressure is proportional to concentration, a law based on PFEFFER'S work a decade earlier, applies to all concentrations whatsoever, without any allowance for electrolytic dissociation or hydration, both of which must play an important rôle in the pressure of these concentrated solutions. On such an assumption the pressure should be near 125 atmospheres.

That this figure is too low can readily be determined from data as to the electrical conductivity of NaCl derived from the Landolt-Börnstein tables. A 4 M solution would exceed 130 atmospheres, so that a saturated solution should run to 160 atmospheres or beyond, merely due to ionization.

Moreover, there is reason for believing that this law of the relation of osmotic pressure to concentration holds only for lower concentrations, and that still further corrections are necessary in the case of highly concentrated solutions; for the actually observed pressures depart widely from the theoretical requirements as concentration increases. BERKELEY and HARTLEY (8) measured the osmotic pressure of solutions of sucrose, dextrose, and galactose by an ingenious method, and have shown that dilute solutions give



pressures agreeing with the gas laws. But curves representing the pressures in higher concentrations show that the osmotic pressure increases in strong solutions much more rapidly than the molecular concentration.

Formerly it was customary to calculate the osmotic pressure on the basis of solution volumes. MORSE, FRASER, and others, however, found a better agreement between increase of concentration and osmotic pressure when the concentration is referred to a liter of solvent rather than to a liter of solution. RENNER (31) has recently given an excellent summary of the literature on the calculation of osmotic pressures, and holds that this change from liter of solution to liter of solvent will bring into agreement the pressures obtained by plasmolytic and those obtained by cryoscopic methods, the former having been in error.

Although making this change brings agreement between theoretical and observed pressures for more concentrated solutions than was true with the old method of making up molecular solutions, the observed pressures are still considerably above the pressures demanded by our theories, after all these corrections and improvements in method have been made.

The relation of the actual pressures found by BERKELEY and HARTLEY to the pressures demanded theoretically by both methods of calculating pressure from concentration is shown graphically by PHILIP (27, p. 53) by means of curves. RENNER also shows a similar diagram in his recent paper but does not mention PHILIP'S discussion. There is little doubt of the correctness of the pressures found by the direct methods of measurement employed by BERKELEY and HARTLEY, as their results have been confirmed recently by TROUTON (36), who uses an entirely new method for the direct measurement. Ether will not take up as much water from a solution as it will from pure water. He determines the pressure necessary to force as much water from a solution of sucrose into ether as the ether will take up normally from pure water (1.05 per cent). The pressure required to force the water content of ether up to 1.05 per cent from a solution of sucrose containing 600 grams per liter was 80 atmospheres. BERKELEY'S result was 81 atmospheres with the same strength of sucrose solution, an agreement well within the limits of error.



The findings of BERKELEY and HARTLEY were discussed by CALLENDAR (13), who offers a plausible explanation for the high pressures observed. He suggests that the solute probably enters into a molecular complex with the solvent, forming hydration compounds. This chemical combination of solute with solvent reduces the number of free molecules of water in the solvent, decreases therefore the vapor tension, or, what amounts to the same thing, increases the osmotic pressure; for, as CALLENDAR says: "There is a definite and simple relation between vapor pressure and osmotic pressure which has been verified by Lord BERKELEY and HARTLEY for strong solutions."

The degree of hydration is represented by  $a$  in CALLENDAR'S discussion, and he believes  $a=5$  in the case of the sucrose solution used by BERKELEY; and in the case of KAHLENBERG'S (23) observations on the rise of boiling-points in concentrated solutions of NaCl, the curve agrees with a hydration value of  $a=6$  up to a concentration in which there are 6 molecules of NaCl present for every 100 molecules of water (CALLENDAR 13, p. 493, diagram p. 492).

CALLENDAR assumes that the molecular complex formed by the hydration is a *definite* chemical compound at any given concentration. In this he does not agree with JONES and BASSETT (22), who claim to have shown that such hydration compounds are indefinite, and may vary from a few to many molecules of water to each molecule of solute in any given concentrated solution.

If the compounds formed were indefinite, it would not be possible to explain the increased osmotic pressure of strong solutions by hydration; but CALLENDAR points out that the determinations of JONES and his co-workers are erroneous at various places, and their conclusions untrustworthy. The writer can only express it as his opinion that CALLENDAR'S assumption is probably the correct one, that the hydration compounds are definite, and that the osmotic pressure of concentrated solutions depends partly upon the hydration value of the molecular complex formed.

If this opinion is correct, then DIETERICI'S and RACIBORSKI'S figures for the osmotic pressure of saturated NaCl solutions, which are based on vapor pressure determinations, are much nearer the truth than BROWN'S figures for the same solution. Although the



difference between the two figures is very large, the former being to the latter as 3:1, inspection of the curves in PHILIP'S little book shows that at a concentration of 700 grams of sucrose per liter of solution, the observed pressure would be to the theoretical pressure calculated on the basis of solution volumes as 3:1. Moreover, the figures of RACIBORSKI for saturated LiCl solutions (80.7 per cent at 20° C.) were obtained by the same methods which DIETERICI used for NaCl; his results give to this solution an osmotic pressure of 965.3 atmospheres. This does not seem too high if NaCl has a value of 375 atmospheres; for NaCl at saturation at 18° C. is a 5.42 M solution, and LiCl a 13.3 M solution (SMITH'S *General chemistry*).

These pressures are of interest on account of their magnitude. If this measure is correct for the osmotic pressure of saturated lithium chloride, then the internal forces causing entrance of water into air-dry *Xanthium* seeds must be at the initial moment in the neighborhood of 965 atmospheres. It is as difficult as it is interesting to think of a saturated aqueous salt solution being as "dry" as an air-dry seed.

From saturated solutions of NaCl these air-dry seeds will imbibe 7 per cent of their air-dry weight. It is readily seen, then, if the pressures given for the two solutions are correct, that the imbibition force in a seed which contains 8-9 per cent of hygroscopic moisture (air-dry) is 965 atmospheres, and that the addition of 7 per cent more water reduces the capillarity and imbibition force from 965 atmospheres to 375 atmospheres, a loss of 590 atmospheres.

The rapid increase of the internal forces with the decreasing water content of the seeds is shown graphically in fig. 8, which is

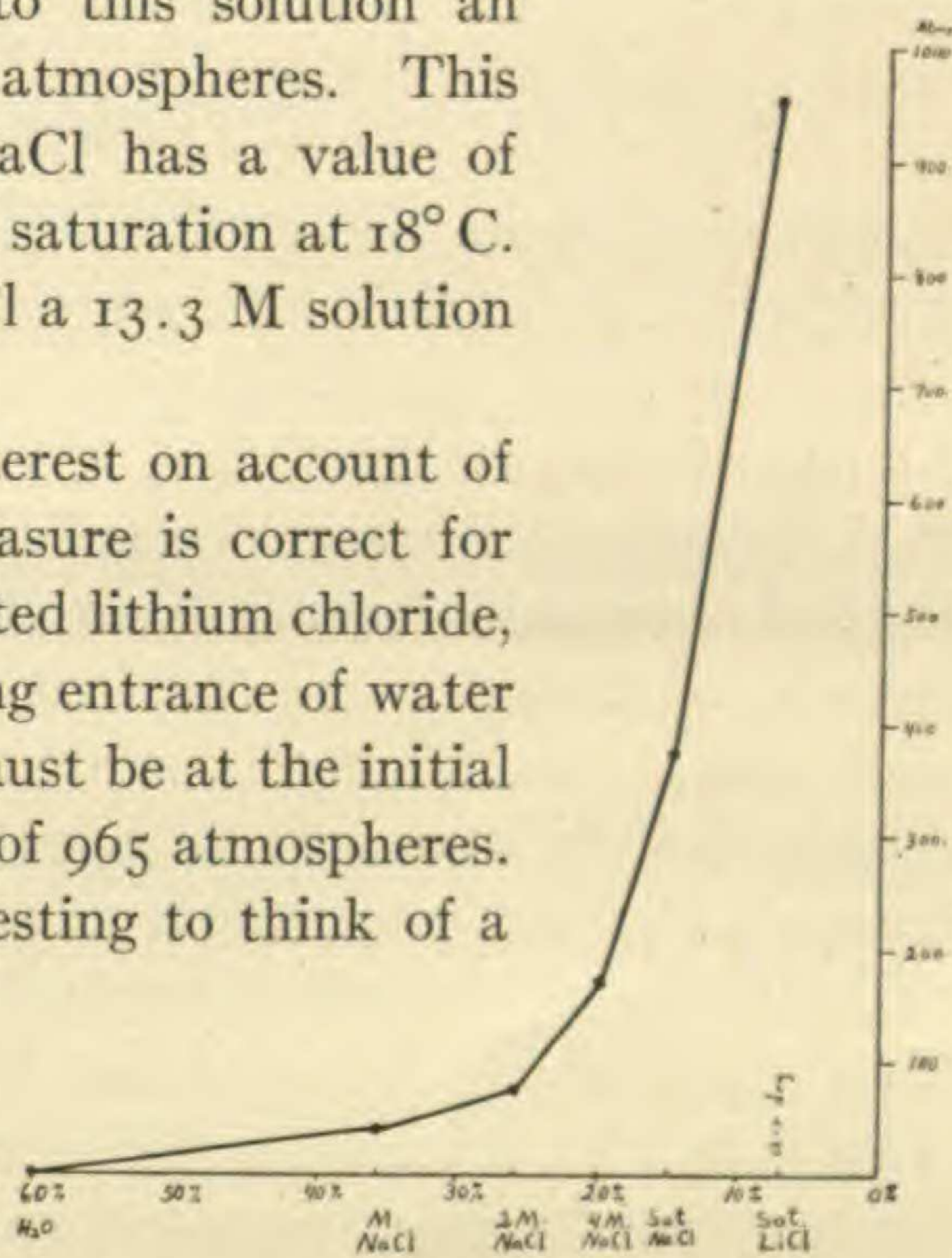


FIG. 8.—Curve of imbibition in M-NaOH, showing the development of osmotically active substances within the semipermeable coat during the second day; the pressure thus developed bursts the outer layers, but leaves the inner one intact.



based upon the figures presented in this discussion. What the initial capillary or surface force would be if all hygroscopic water could be eliminated without changing the fundamental nature of the embryo must be left purely to conjecture; but the figures would probably exceed those which RODEWALD found for starch. He states that dry starch on swelling develops a pressure of 2523 atmospheres (32, p. 227). At present we have no adequate means of measuring such large forces.

### III. Discussion

The results here recorded have a bearing upon many problems in widely separated fields of research. It will be impossible to consider every phase of the subject, and only the more important matters will be considered here.

The general occurrence of non-living semipermeable membranes in plant structures, especially as seed and fruit coats, is of the greatest interest. A large amount of work has been done upon seeds with coats intact, especially upon seeds showing delayed germination, in attempts to stimulate protoplasm to activity. FISCHER (16) used many different kinds of acids, alkalies, and salts on seeds. LEHMANN (26), BECKER (6), and many others have used Knop's solution, etc., as stimulants for resting seeds. In BECKER'S paper especially the seeds used by him, mostly Compositae, show such peculiar irregularities of behavior in germination that one cannot escape the conviction that the coats are responsible for much of it. If semipermeability of protective structures is as common as now appears to be the case, much of the work done with salts and other substances acting through the coats will lose considerably in its significance. The very salts which are assumed to enter the seed may be excluded by the testa of the seed. No safe conclusions can be drawn from studies in which the physical characters of the testa are not definitely known. This discovery only emphasizes what the writer has said elsewhere (34) regarding the necessity of removing seed coats when the properties of embryos are being investigated. The testa has physical and chemical characters which may enable it to modify greatly any factor entering into germination behavior, and the effects of these characters must be known before



any sound conclusions can be drawn. Semipermeability is now shown to be common enough that its existence should be proved or disproved before proceeding to use stimuli acting through membranes such as those referred to here.

With a number of the solutions used in these experiments a very high percentage of increase in weight was noticed, running much higher than the imbibition in pure water. This was noticed with  $\text{HgCl}_2$ ,  $\text{AgNO}_3$ , several alcohols, acetic acid, and especially the alkalis. The intake in these cases cannot be considered as due entirely to imbibition and capillarity. Several things may be responsible for the large increase in weight. In the case of the solutions of the heavy metals their specific gravity has something to do with the great increase; but there is convincing evidence that this is not the only cause.

The seeds become greatly swollen, until in many instances they are perfectly cylindrical and stretched to inordinate size. In these cases a fluid is found between the coat and the rest of the seed. Evidently there is a dissolution of various constituents of the embryo whose decomposition products are osmotically active, which exert great pressure upon the coat from within, and which thus cause a very large intake of water. This is true especially when alkalis are used. Fig. 9 shows the curve of increase in weight of seeds kept in molecular  $\text{NaOH}$ . The earlier part of the curve, up to 24 hours, resembles the imbibition curve for water, only the entrance is a little more rapid. Then suddenly the weight increases under the pressure of dissolved organic substances from within, and by the end of three days the outer layers of the coat have been bursted, leaving the inner layer intact. Through its translucent cells one can see the

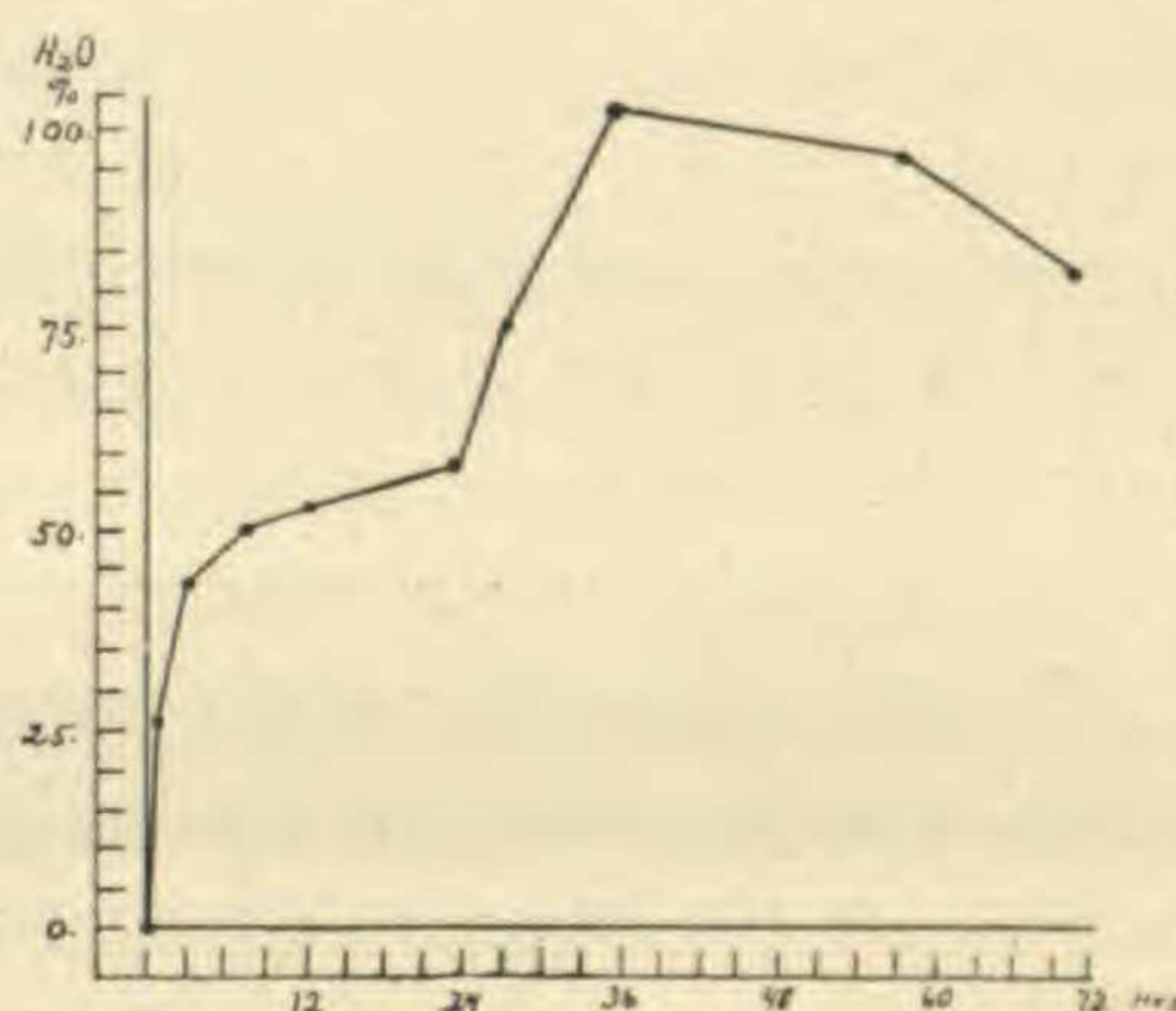


FIG. 9.—Curve showing increase of imbibition force in seeds with decrease of water content; percentage of  $\text{H}_2\text{O}$  is based on absolute dry weight; air-dry seeds are considered to have 8 per cent of hygroscopic moisture in this diagram; the force as here plotted is based on figures discussed in the text.



embryo, not much above normal in size, surrounded on all sides by a liquid which is held in by the semipermeable membrane.

This curve is of great interest in connection with FISCHER'S (16) work on the seeds of water plants. When the seeds have a brittle instead of an elastic coat, the pressure from within may be great enough to rupture the coat, and allow oxygen or some other requisite for germination to enter. There is no doubt that this actually happens with acids and alkalies in the case of *Alisma Plantago-aquatica*, one of the seeds which FISCHER stimulated with hydrogen and hydroxyl ions, and which CROCKER (15) showed at the same time needed only to have the coat broken to bring about germination. In view of the effect of certain acids and alkalies on seeds with semipermeable coats, the reason for germination on treatment with these substances is obvious.

In this connection sight must not be lost of the possible effect of acids, alkalies, etc., on the colloids of the seed. FISCHER (17, 18) has shown the remarkable effect of acids and alkalies on the water-holding power of fibrin and frog muscle, and has pointed out the relation of acids to edema and glaucoma (19). A part of the increased weight may be due to the colloids in the embryo holding more water in the different states of ionization induced in them by the presence of acids and alkalies; but most of it is certainly due to the osmotic pressure from within, which adds a large quantity beyond what capillarity and imbibition would take up.

The behavior of the coat in retaining dissolved substances adds further evidence to that already brought forward to show that even after prolonged treatment in tannin solvents the coats are still semipermeable.

The high internal forces in dry seeds is interesting in connection with recent work on the osmotic pressures of the cells of desert plants. FITTING (20) shows that desert vegetation has in many instances cell sap with more than 100 atmospheres of pressure, an adaptation which is of great importance to the plant whose "saturation deficit" must withdraw the water from an arid soil. The value of FITTING'S work would have been enhanced if he had used boiling or freezing-point methods as a check to the plasmolytic methods of determining the osmotic pressure. The possibility suggests itself



that the forces which extract water from the soil particles and move it through the plant may not be entirely osmotic whenever the force needed runs high, but may involve capillarity and imbibition as well. A study of surface tension forces in soils should throw some light on this subject.

Many theories have been advanced to explain the cause of semipermeability. Chief among these are (1) the filter or sieve theory, (2) the solubility theory, (3) the "Haftdruck" theory of TRAUBE, and (4) the hydrone theory of ARMSTRONG. Each theory has a certain amount of supporting evidence, and any of them could be applied to the problems of physical or physiological semipermeability.

Since ARMSTRONG (3) used the hydrone theory to explain the semipermeability of the coat of *Hordeum vulgare* as described by BROWN, I have given it more attention than the others, not that it seemed to offer the best explanation, but because of its weakness.

ARMSTRONG'S conception of water as a mixture of hydrone, hydrol, and hydronol was developed several years ago (1, 2, 4). The hydrone compounds are composed of  $=\text{OH}_2$ , which is related to the hydrones much as  $=\text{CH}_2$  is to the carbon compounds. The more complex molecules, as penthdrone, are believed to be inert, resembling the polymethylenes in this respect; whereas the simple hydrones are much more active.

The size of the hydrone molecule is believed to be influenced by temperature. As the temperature of water rises, the complex hydrones are supposed to split up into simpler molecules, which renders the water much more active in all its relations. This conception has received some support recently from the work of BROWN and WORLEY (12), who studied the relation of temperature to the rate of water imbibition by seeds of *Hordeum vulgare*.

Their results indicate that the velocity of water intake may be a logarithmic function of the temperature. The temperature coefficient for the imbibition rate follows approximately the VAN'T HOFF law, indicating that chemical processes are involved in water intake. They suggest that the chemical change thus indicated is the simplification of the complex hydrone compounds into smaller, more active molecules.



Certain solutes are believed to have the same, or similar, effects upon the hydrones as increase in temperature. For instance, acetic acid, and other substances mentioned in this paper as leading to supernormal imbibition, may increase the rate of entrance by breaking down the inert complex molecules of water. If the interpretation of the results of BROWN and WORLEY is correct, the measure of the rate of water intake may be looked upon as a measure of the activity of water when in a certain state which depends on temperature, or on the presence of certain solutes. While this work is of the greatest interest, it is too early to make any general applications of their results.

If the writer understands ARMSTRONG'S application of his hydrone theory (3) to semipermeable membranes, the fine particles which make up the membrane are assumed to be chemically united to hydrone or hydrol, perhaps under the influence of surface force. All the intramolecular passageways through the membrane are therefore guarded by hydrone elements. When a salt goes into solution the solute molecules also are believed to be hydrolated in case of non-penetrating solutes. Now if a hydrolated salt presents itself for passage through a hydrolated or hydronated membrane, the salt is seized and held back by the mutual attraction of the hydrolated surfaces of the salt and membrane. If on the other hand an unhydrolated salt presents itself to the same membrane the hydrolated passageways are indifferent to the salt, and it passes through the membrane without the "chemical seizure" retarding its entrance. Selective action would depend upon the salt rather than upon the membrane, for an unhydrolated membrane should allow all salts to pass providing other physical and chemical conditions necessary to passage were met.

The penetrating salts, whether electrolytes or non-electrolytes, are conceived to be those which can exist in water solution in unhydrated condition, or which attract water only to a slight extent; whereas those which are excluded are those which form hydration compounds of considerable stability; and the semipermeability depends on the mutual attraction of hydrated salt and hydrated membrane.

It is the writer's opinion that ARMSTRONG'S rather fanciful



theory of the structure of water has not been well received by American chemists; perhaps it has not been given the attention it deserves. As regards its application to the problems of semipermeability there seems to be some difficulty. The data obtained at one point with *Xanthium* seeds argue against the hydrone theory as a universal explanation.

For instance, BROWN found that both NaCl and AgNO<sub>3</sub> failed to penetrate the coat of *Hordeum*. ARMSTRONG says in effect that the membrane is hydronated, both salts are hydronated, therefore they do not enter, although the water in which they are dissolved may enter freely. In my experiments NaCl does not pass through the *Xanthium* coats, therefore both salt and membrane must be hydronated. Now if ARMSTRONG'S theory is correct, a solution of AgNO<sub>3</sub> should also be excluded, for BROWN'S experiments have shown that AgNO<sub>3</sub> is a hydronated salt when in solution. But this does not happen, for silver nitrate is retarded but slightly, whereas according to ARMSTRONG'S hypothesis the mutually hydronated surfaces should attract each other so strongly as to prevent the passage of the salt. Trichloroacetic acid was exceptional in its behavior in BROWN'S experiments, so that the hydrone theory cannot as yet be accepted as a general explanation of selective semipermeability. More facts are needed before the physiological semipermeability of living or the physical semipermeability of non-living membranes can be adequately explained.

Further studies on the seed coats of *Xanthium* and other seeds are being continued. The microchemistry of the coat, its relation to oxygen diffusion in respiration, its relation to the impermeability of "dry" solvents, and its use as a measure of surface tension forces in soils are now under investigation. The earlier results of these studies cannot of course be presented at this time.

#### IV. Summary

1. The dry seed coats of *Xanthium* are impermeable to dry alcohol, ether, chloroform, and acetone. BECQUEREL'S results with the coats of other seeds are confirmed.

2. Evidence of the diffusion of oxygen through absolutely dry seed coats was not obtained.



3. Selective semipermeability like that found in *Hordeum* has been demonstrated for the seed coat of *Xanthium*.

4. The following substances seem to be excluded: NaCl, CuSO<sub>4</sub>, K<sub>2</sub>CrO<sub>4</sub>, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, glycerol, sugars, HCl, tartaric acid.

5. The following enter, either slowly or rapidly: NH<sub>4</sub>NO<sub>3</sub>, AgNO<sub>3</sub>, NaNO<sub>3</sub>, KNO<sub>3</sub>, KCl, HgCl<sub>2</sub>, FeSO<sub>4</sub>, alcohols, ether, iodine, KOH, NaOH, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, acetic acid, lactic acid, citric acid.

6. The selective activity is independent of any living substance in the seed coat.

7. The coat of *Xanthium* can be removed and used as an osmotic membrane, possessing a great advantage over the coats investigated by BROWN and SCHRÖDER.

8. The testa is composed of three layers, the outer of which cannot function as a semipermeable membrane. The middle layer is several cells thick, the inner layer one cell thick except in the chalazal region. This last layer is probably the nucellar epidermis.

9. By use of strong alkalies the inner membrane can be split loose from the middle layer. Both layers possess osmotic properties, the inner layer in a higher degree than the middle one.

10. Neither layer is as efficient alone as when both are left together. The impairing of the membranes may be due to stretching, or to the effects of the macerating agent.

11. The inner layer is nearly pure cellulose, unsubscriberized, but perhaps containing some tannin. The middle coat contains more tannin than the inner coat.

12. The tannin does not form a continuous layer in the seed coat. Moreover, treatment with tannin solvents does not destroy semipermeability. The evidence is adverse to REICHARD'S view that semipermeability is due to tannin compounds.

13. Semipermeability has been demonstrated for the seed coats of a number of plants in six widely separated families. Many dead plant membranes may possess this property.

14. The capillary and imbibition force of the embryo of *Xanthium* as measured by the osmotic pressure of concentrated salt solutions is about 965 atmospheres when the seed is air-dry.

15. An increase in the moisture of the embryo equal to 7 per cent of its air-dry weight reduces the internal forces by 590 atmospheres.



16. The unusual intake of water noticed with certain substances, especially with certain acids and alkalies, is due largely to the development of osmotically active substances inside the semi-permeable membrane.

17. There is some evidence unfavorable to ARMSTRONG'S hydrone theory of selective semipermeability.

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